CLAIMS

1. A method for the removal of a substance carrying a negative charge and being present in an aqueous liquid (I), said method comprising the steps of

(i) contacting the liquid with a matrix carrying a plurality of ligands comprising a positively charged structure (anion-exchanger) and a hydrophobic structure under conditions permitting binding between the ligands and the substance, and

(ii) desorbing said substance from said matrix,

characterized in that

(I) each of said ligand plus a spacer has the formula:

-- SP---[Ar-R_1-N^+($R_2R_3R_4$)]

where

(A) [Ar-R_1-N^+($R_2R_3R_4$)] represents a ligand in which

a) Ar is an aromatic ring,

b) R_1 is $[(L)_nR'_1]_m$ where

- n and m are integers selected amongst zero or 1;
- L is an amino nitrogen, an ether oxygen or a thioether sulphur;
- R'_1 is a bivalent linker group selected among
  1) linear, branched or cyclic hydrocarbon groups;
  2) -C(=NH)-;

c) $R_{2\text{-}4}$ are selected among hydrogen and lower alkyls;

(B) SP is a spacer providing a carbon, a nitrogen, a sulphur or an oxygen directly attached to Ar-R_1-N^+($R_2R_3R_4$);

(C) --- represents that the spacer is replacing a hydrogen in (Ar-R_1-N^+($R_2R_3R_4$);

(D) -- represents binding to the matrix; and

(II) desorption in step (ii) is carried out under anion-exchange conditions when the substance is a serine protease and in particularly when R'_1 = -C(=NH)-.

2. The method of claim 1, characterized in that anion-exchanger (1) is capable of

(a) binding to the substance of interest in an aqueous reference liquid (II) under anion-exchange condition at an ionic strength corresponding to 0.3 M NaCl and,

(b) permitting a maximal break through capacity in the pH interval 2-12 for the substance ≥ 200 %, such as ≥ 300% or ≥ 500% or ≥ 1000 %, of the maximal break through capacity in the pH-interval 2-12 of the substance for Q-Sepharose Fast Flow (Amersham Pharmacia Biotech, Uppsala, Sweden),

said anion-exchangers having essentially the same ligand density and break through capacities being determined under the same conditions.

3. The method of any of claims 1-2, characterized in that m = 1 and R'_1 is a bivalent linker group selected among linear, branched or cyclic hydrocarbon groups that may be substituted and/or have a carbon chan that is interrupted by ether oxygen, thioether sulphur or amino nitrogen.

4. The method according to any of claims 1-3, characterized in that the matrix with its plurality of ligands has a pKa ≤ 12 and/or is a primary or secondary nitrogen.

5. The method of any of claims 1-4, characterized in that at least one of Ar, SP, R'_1 and R_{2-4}, comprises one or more electron acceptor-donor atoms or groups at a distance of 1-7 atoms from the positive nitrogen in $-N^+(R_2R_3R_4)$, preferably said acceptor-donor atoms or groups participating in hydrogen-bonding, and with the proviso that for Ar this atoms or groups are not sp^2-carbons in an aromatic structure.

6. The method of any of claims 5, characterized in that said

(i) electron donor-acceptor interaction is hydrogen bonding and/or

(ii) donor atoms/groups are selected among:

(a) oxygen with a free pair of electrons, such as in hydroxy, ethers, carbonyls, and esters (-O- and -CO-O-) and amides,

(b) sulphur with a free electron pair, such as in thioether (-S-),

(c) nitrogen with a free pair of electron, such as in amines, amides including sulphone amides,

(d) halogen (fluorine, chlorine, bromine and iodine), and

(e) sp- and sp^2-hybridised carbons; and/or

(iii) acceptor groups are selected amongst groups that consists of a electron-deficient atom such as hydrogen and/or an electronegative atom.

7. The method of any of claims 5-6, characterized in that at least one of said one or more hydrogen-bonding atoms is present as a branch group in SP or as a part of the chain in SP extending from the base matrix to the ligand.

8. The method according to any of claims 1-7, characterized in that SP contains

(a) a carbon atom with preference for a carbonyl carbon or an sp^3-hybridised carbon; or

(b) a nitrogen atom with preference for an amino or an amido nitrogen; or

(c) a sulphur atom with preference for a thioether sulphur atom; or

(d) an oxygen, with preference for an ether oxygen atom;

which is directly attached to the ligand $Ar\text{-}R_1\text{-}N^+(R_2R_3R_4)$, with the proviso that items (b)-(d) only apply when the spacer binds to Ar or R_1.

9. The method of any of claims 1-2, characterized in that n = 0, m = 1, R'_1 = -C(=NH)-, $R_{2\text{-}4}$ = hydrogen, Ar = p-C_6H_4-, SP is attached to Ar via a secondary amino nitrogen, such as -NH-.

10. The method of any of claims 1-9, characterized in that the ionic strength during the adsorption/binding step (i) is larger or equal with the ionic strength of 0.25 M NaCl water solution.

11. The method of any of claims 1-10, characterized in that the pH of aqueous liquid (I) is $\leq$ pKa + 2, such as $\leq$ pKa + 1, of the anion-exchanger or of an anion-exchanger ligand present in the anion-exchanger.

12. The method of any of claims 1-11, characterized in that the pH of aqueous liquid (II) is different from the pH of aqueous liquid (I) in order to decrease the negative charge of the substance.

13. The method of any of claims 1-12, characterized in that the polarity of aqueous liquid (II) is lower than the polarity of aqueous liquid (I).

14. The method of any of claims 1-13, characterized in that a structural analogue of $Ar\text{-}R_1\text{-}N^+(R_2R_3R_4)$ is present in aqueous liquid (II) in a larger concentration than in aqueous liquid (I).

15. An anion-exchanger (1) comprising a plurality of anion-exchange ligands each of which is attached via a spacer to a hydrophilic base matrix, characterized in that

(a) the ligands plus their spacers comply with the formula:

$$\text{--SP---[Ar-}R_1\text{-}N^+(R_2R_3R_4)\text{]}$$

where the symbols have the same meaning as in any of claims 1-10, and

(b) the anion-exchanger (1) has a maximal breakthrough capacity in the pH-interval 2-13 for at least one reference proteins selected amongst ovalbumin, conalbumin, bovine serum albumin, β-lactglobulin,α-lactalbumin, lyzozyme, IgG, soybean trypsin inhibitor (STI) which is ≥ 200%, such as ≥ 300% or ≥ 500% or ≥ 1000% of the maximal breakthrough capacity in the pH-interval 2-12 obtained for a Q-exchanger ($\text{-}CH_2CH(OH)CH_2N^+(CH_3)_3$) (anion-exchanger 2), the support matrix, degree of substitution, counter-ion and running conditions being the same for anion-exchanger (1) and anion-exchanger (2).

16. The anion-exchanger of claim 15, characterized in that the relative break-through capacity is measured under anion-exchanger condition.

17. A method for testing (screening) the appropriateness of one or more anion-exchangers for removing a substance from a liquid, said method comprising the steps:

(a) providing a library which comprises

(i) one or more anion-exchangers to be tested (exchangers 1, 2, 3, 4 . . . . . n; n = an integer > 0) each of which anion-exchangers differs with respect to kind of ligand (ligands 1, 2, 3, 4, ...n), and

(ii) a reference anion-exchanger having a reference ligand, the support matrix etc being essentially the same in the exchangers 1, 2, 3, 4 . . . .n and in the reference anion-exchanger;

(b) determining the maximal breakthrough capacity in the pH-interval 2-12 of exchanger 1 for the substance at a predetermined condition;

(c) determining the maximal breakthrough capacity in the pH-interval 2-12 of the reference anion-exchanger for the substance at the same condition as in step (b);

(d) concluding with the aid of the relation between the maximal breakthrough capacities obtained in steps (b) and (c), if anion-exchanger 1 is appropriate to use for removing the substance; and

(e) repeating, if necessary, steps (b)-(c) for at least one of the exchangers 2, 3, 4 . . . n.

18. The method of claim 17, characterized in that the steps (b) and (c) are carried out under anion-exchanger conditions.

19. A method for removing salt from a negatively charged substance, preferably amphoteric, when present in a solution (liquid (I)), which method comprises the steps of:

(i) contacting liquid (I) liquid with an anion-exchanger (1) that comprises a base matrix carrying a plurality of ligands in which there is a positively charged nitrogen under conditions permitting binding between the anion-exchangerr and the substance,

(ii) desorbing said substance from said anion-exchanger by the use of a liquid (liquid (II)).

characterized in:

(A) selecting anion-exchanger (1) among anion-exchangers that are

(a) capable of binding the substance of interest in an aqueous reference liquid at an ionic strength corresponding to 0.25 M NaCl; and

(b) permitting a maximal breakthrough capacity in the pH interval 2-12 for the substance ≥ 200 %, such as ≥ 300% or ≥ 500% or ≥ 1000 %, of the

breakthrough capacity of the substance for Q-Sepharose Fast Flow (anion-exchanger 2, Amersham Pharmacia Biotech, Uppsala, Sweden), said anion-exchangers having essentially the same ligand density and the breakthrough capacities being determined under the same conditions;

(B) adjusting the pH of liquid (II) in step (ii) by the use of an acid-base pair to a value that means a lower net positive charge on-the anion-exchanger and/or a lower net negative or positive charge on the substance thereby enabling elution at a lowered ionic strength compared to liquid (I).

20. The method of claim 19, characterized in that at least one member of the acid-base pair buffer has a vapour pressure that is higher than the substance.

21. The method of any of claims 19-20, characterized in that the substance in the liquid of low salt content obtained in step (ii) is ionized in a mass spectrometer.